



NIEHS Users Group Meeting

Sample Submission and Recent Advances in the Microarray Center

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Outline for Today's Talk

- Website Updates
- RNA Isolation and Submission
- RNA Labeling Methods
- High Throughput Sequencing
- Scanning and Image Analysis Improvements
- Printing Modifications
- The New Human Oligo Chip



Website Updates



NIEHS Microarray Center

National Institute of Environmental Health Sciences

[Background](#)[Methods](#)[Equipment](#)[Bioinformatics](#)[Chips & Clones](#)[FAQs](#)[Example Data](#)[Additional Resources](#)[Collaboration](#)[Contacts](#)[Home](#)



Recent developments in genome sciences have led to the development of DNA microarray technology, a tool of unprecedented power for the study of gene sequence, structure, and expression. Using cDNA microarrays, the expression of thousands of genes can be monitored simultaneously in two biological samples of interest, and the expression patterns compared.

The **National Institute of Environmental Health Sciences** has established a cDNA microarray center which provides access to this technology for the intramural and extramural community. Contained within this web site and linked sites, is a description of the technology as it exists at the NIEHS, how microarray technology can be applied to studies in environmental health, some sample data, as well as proposal and sample submission forms for interested investigators.

NIEHS Contact: **Microarray Center**
Last Modified: 02/15/2002
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- New Look
- More Protocols
- PDF Format
- FAQs
- Search Features

Dir.niehs.nih.gov/microarray



RNA Isolation and Submission



NIEHS Microarray Center

National Institute of Environmental Health Sciences

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Methods and Protocols

The following PDF files require [Adobe Acrobat Reader](#) for viewing.

| |
|---|
| RNA |
| RNA Preparation |
| RT Labeling from Total RNA Source & Qiagen Clean-Up |
| Agilent Bioanalyzer |
| Tissue Collection |
| PRINTING |
| Processing of Slides Prior to Printing |
| Processing of Slides Post-Printing |
| QA/QC of Prints |
| POPO3 DNA Staining for Microarray Slides |

- Tissue Culture
- Liver, Kidney, Thymus, Lung, Prostate, and Ovary
- Skin
- Poly A selection coming soon



RNA Isolation and Submission

Sample Submission Requirements

(samples will be returned if all criteria are not met):

Please check boxes when criteria are met.

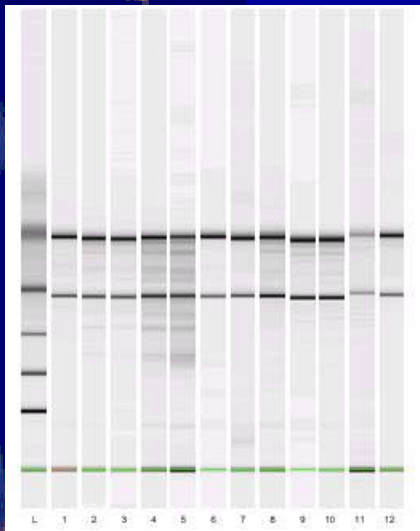
- ☐ a. RNA concentration of a minimum: 5 ug/ul for total RNA or 0.5 ug/ul for Poly A +RNA, in nuclease-free water.
- ☐ b. Minimum submission of 200 ug total RNA or 15 ug Poly A + RNA per comparison (i.e. if a sample, such as a control, is to be compared to more than one other sample, there must be 200 ug total RNA per comparison). Reserve sufficient quantity for signal validation by traditional methods, e.g. northern blot.
- ☐ c. OD 260/280 absorbance ratio of 1.7-2.1, quantitated **using NMC RNA quantitation protocol**. Please see website for current protocols: <http://dir.niehs.nih.gov/microarray/>
- ☐ d. A 5 uL sample at 100ng/ul of each RNA sample submitted for RNA quality check on Agilent Bioanalyzer.
- ☐ e. Submission must be accompanied by **original** gel image (clearly labeled and easily distinguishable) with 1-5 ug/lane of each total RNA species. If submitting mRNA, the gel need only be of the total RNA before poly A selection. Greater than 50% of EtBr stained material must be 28S and 18S bands.
- ☐ f. Screw-cap tubes (with O-ring) neatly labeled with sample name, date, and RNA concentration.
- ☐ g. Tubes shipped inside 50 mL conical tubes on dry ice.
- ☐ h. Completion of **ALL** tables in this form.

- New RNA amounts
- Clear Labeling
- Diluted Sample for Bioanalyzer
- Original Gel Image

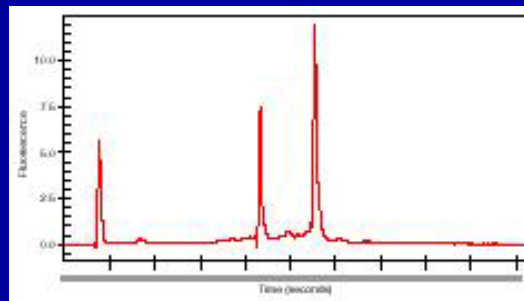


RNA Isolation and Submission

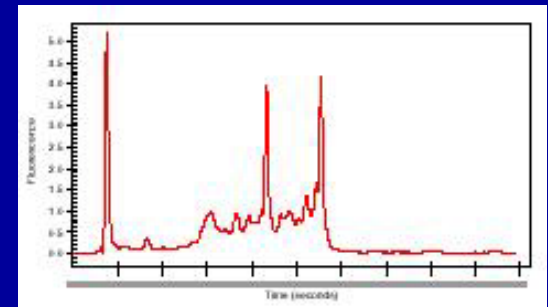
- Agilent 2100 Bioanalyzer
- Measure RNA quality and quantity
- Uses small sample size and take minutes



Agilent Gel Image



Good Quality RNA



Degraded RNA



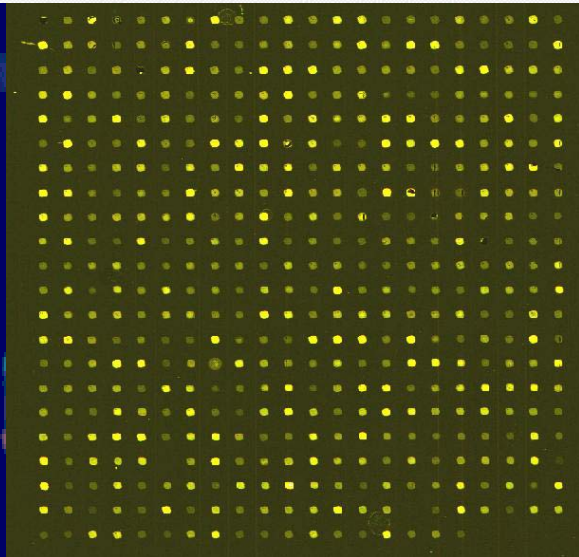
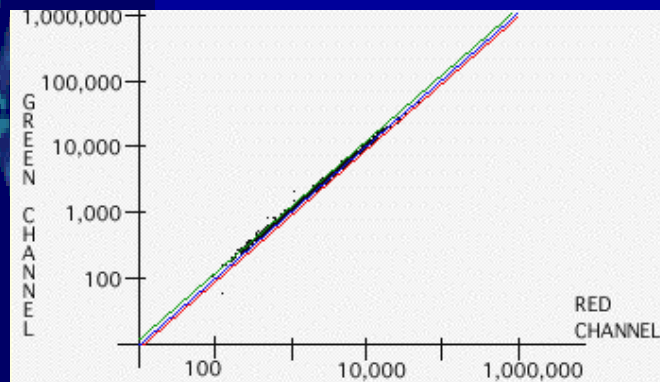
RNA Labeling Methods

- Direct Labeling
 - 25-50 ug per Channel
 - Depends on RNA Quality
- Indirect Labeling
 - Advertises 5-20 ug per Channel
- RNA Amplification
 - Advertises 1-10 ug per Channel
- Dendrimer Probes
 - Advertises 1-2 ug per Channel

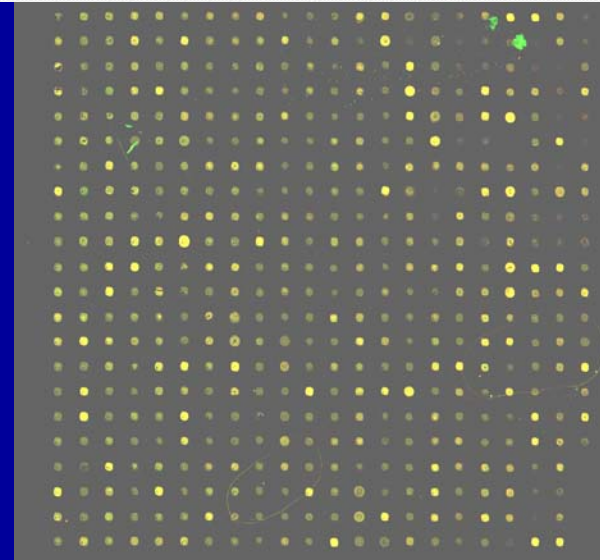
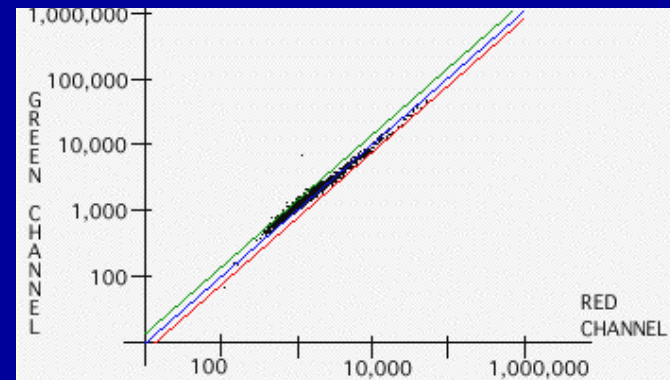


RNA Labeling Methods

Direct Labeling 35 ug



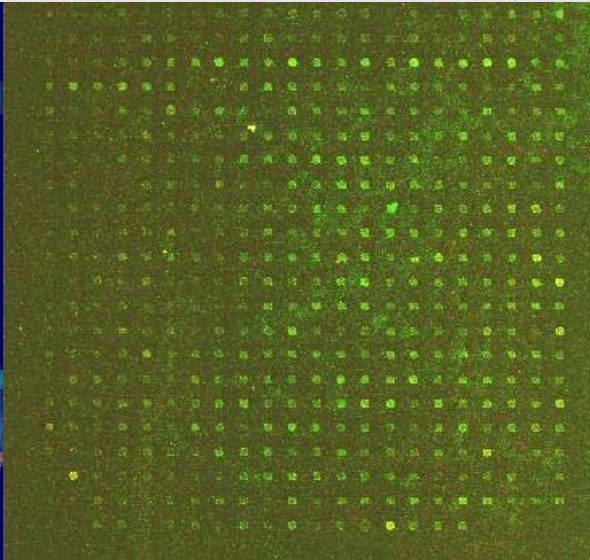
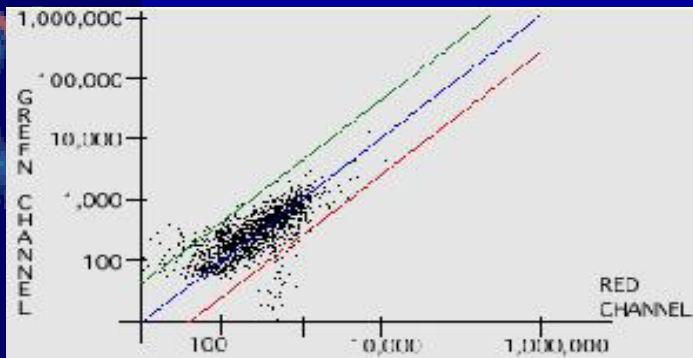
Indirect Labeling 20 ug





RNA Labeling Methods

Dendrimer Probes

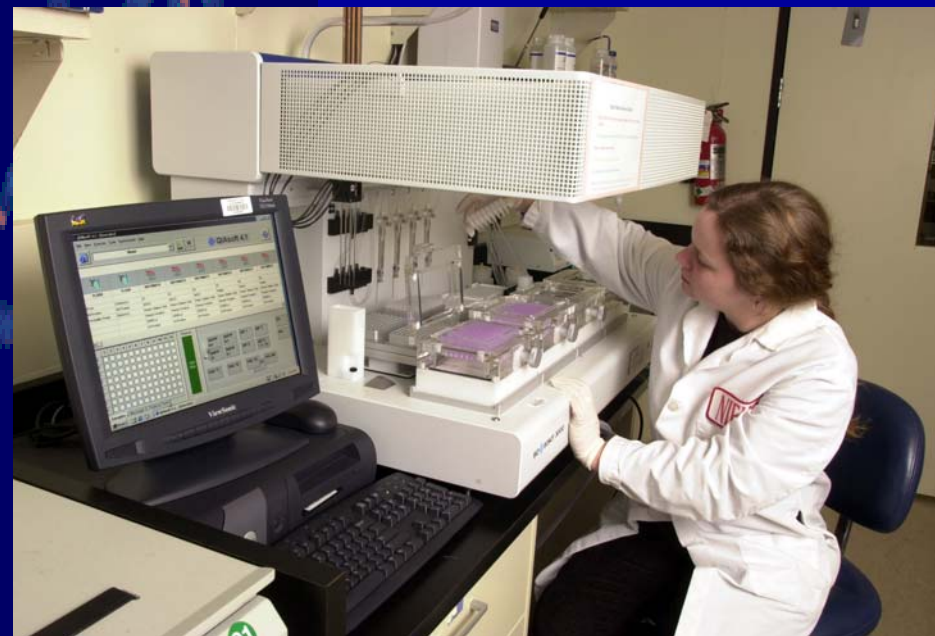


RNA Amplification

This is on the
“To Do List”



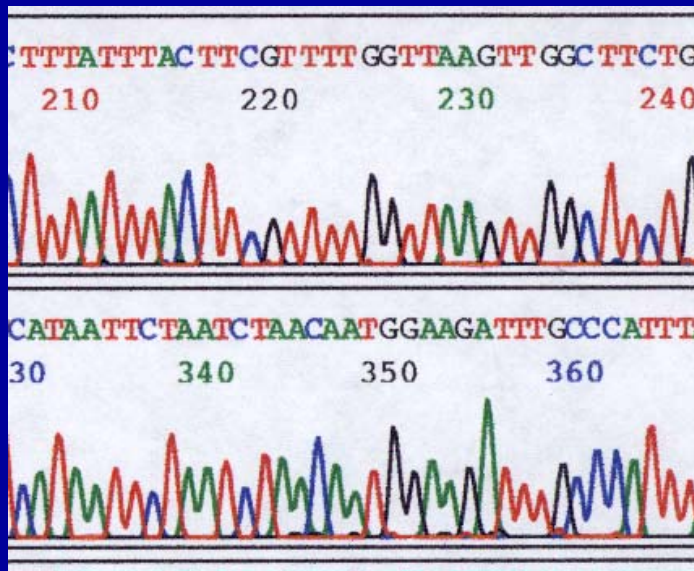
High Throughput Sequencing



- Human Clones 85.22%
- Rat Clones 95.83%
- Mouse Clones 75.0%



High Throughput Sequencing



Using the Qiagen Biorobot we are currently sequencing about 400 clones per week.



Scanning and Image Analysis

NIH Scanner



2 Hours per Slide

Axon



20 minutes per slide



Scanning and Image Analysis



- 5 um Resolution
- 7 minutes per slide
- 48 slide capacity
- Confocal Scanning with Autofocus
- Automated Image Analysis coming soon

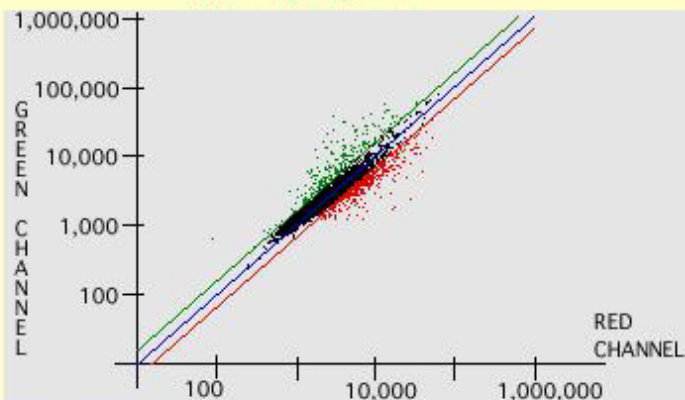


Scanning and Image Analysis

ArraySuite 2.0

- Handles 32 pins
- Quality Metric
- Linear Regression
- Adaptive Confidence Limits
- Easier Gridding

Image Name: p60s83_635_grid.tif
p60s83_532_nm.tif



☒ Log Scale
☒ Calibrated Result

View As:

☐ Histogram
☒ Scatter Plot

Data from:

☒ All targets
☐ Control targets

Calibration by:

☐ Internal Controls
☒ All targets
☐ Background

Background Correction:

☐ Negative Control
☐ Estimation

Normalization Method:

☒ Ratio Distribution
☐ Log-Normal
☐ Linear Regression

Ratio Confidence Interval:

Confidence Level: 99.00

Use CI from: Lower Limit Upper Limit
☒ Int. Controls 0.66 1.52

☐ Adaptive Confidence Interval

Data Filtering By:

☐ Intensity:
☐ Target Size From:
☐ Signal-to-Noise Ratio:
☒ Measurement Quality:
☐ User flagged.

At Least

500
70
0.00
0.01

Ratio Stats

CV = 0.112
M = 1.204

Save DataSheet

Ratio Outliers

Refresh

Refresh dataSheet

Default Setting

Exit



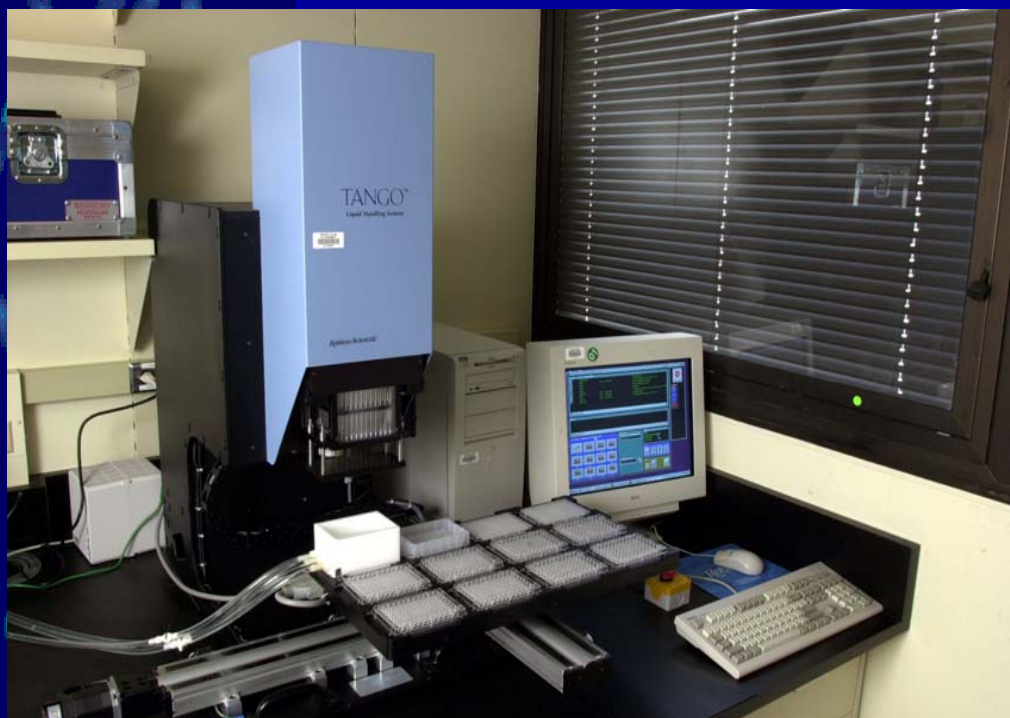
Printing Modifications



- Beecher Instruments
- 96 Slides
- Tortoise and the Hare



Printing Modifications



Making our Tortoise a Hare

- Accurate at small Volumes
- Used for Print Plate Refreshes
- Used for 96 to 384 plate conversions



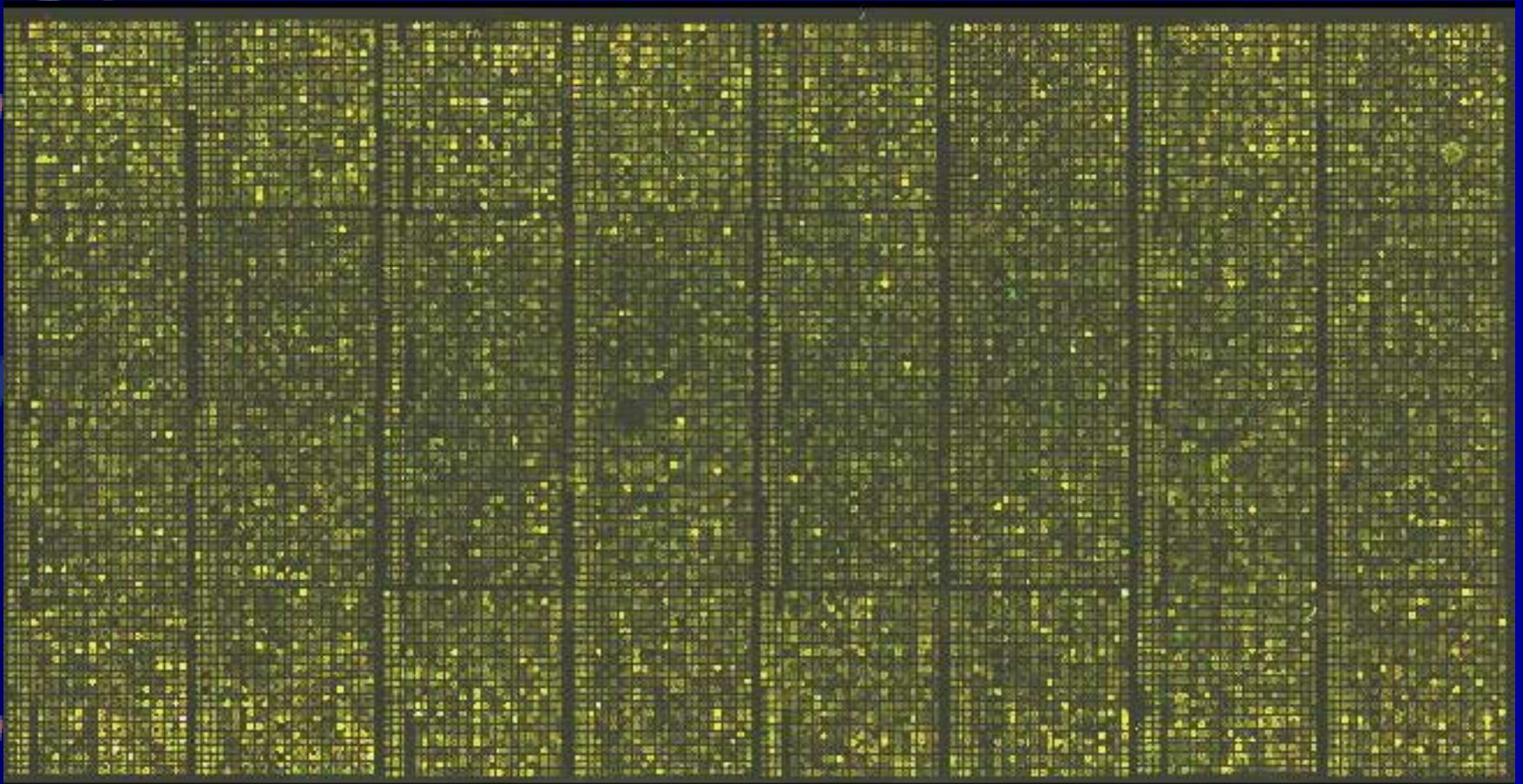
Printing Modifications



- 32 pin Print Head
- Allows use of 384 printing plates
- Basin Pin Wash
- New Vacuum Valve
- Can print 20,000 per slide spots in 1 week

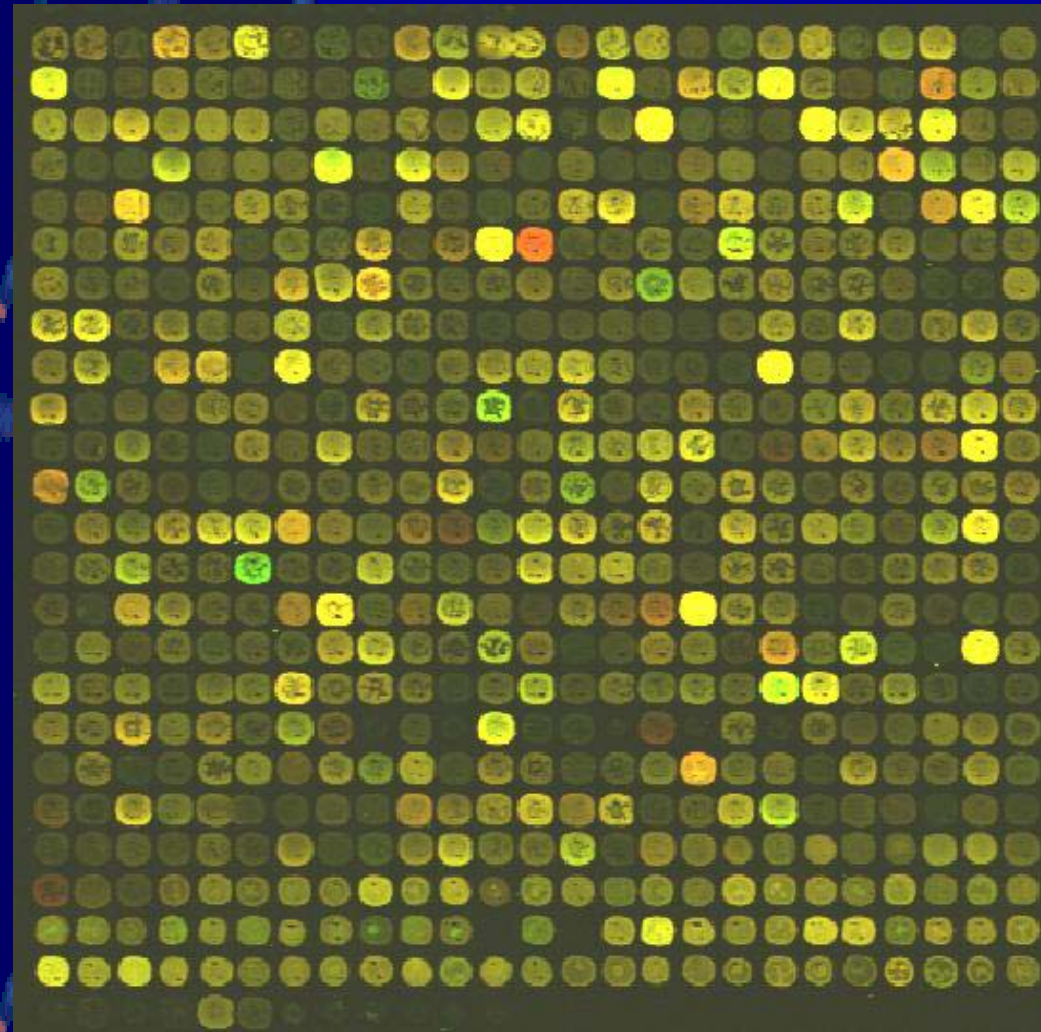


The New Human Oligo Chip





The New Human Oligo Chip



- 17,000 70mer Oligos from Operon
- 2,400 cDNA from the NIEHS ToxChip
- 200 custom add ons from NCI and NIEHS
- The First to add cDNAs and Oligo to the same chip

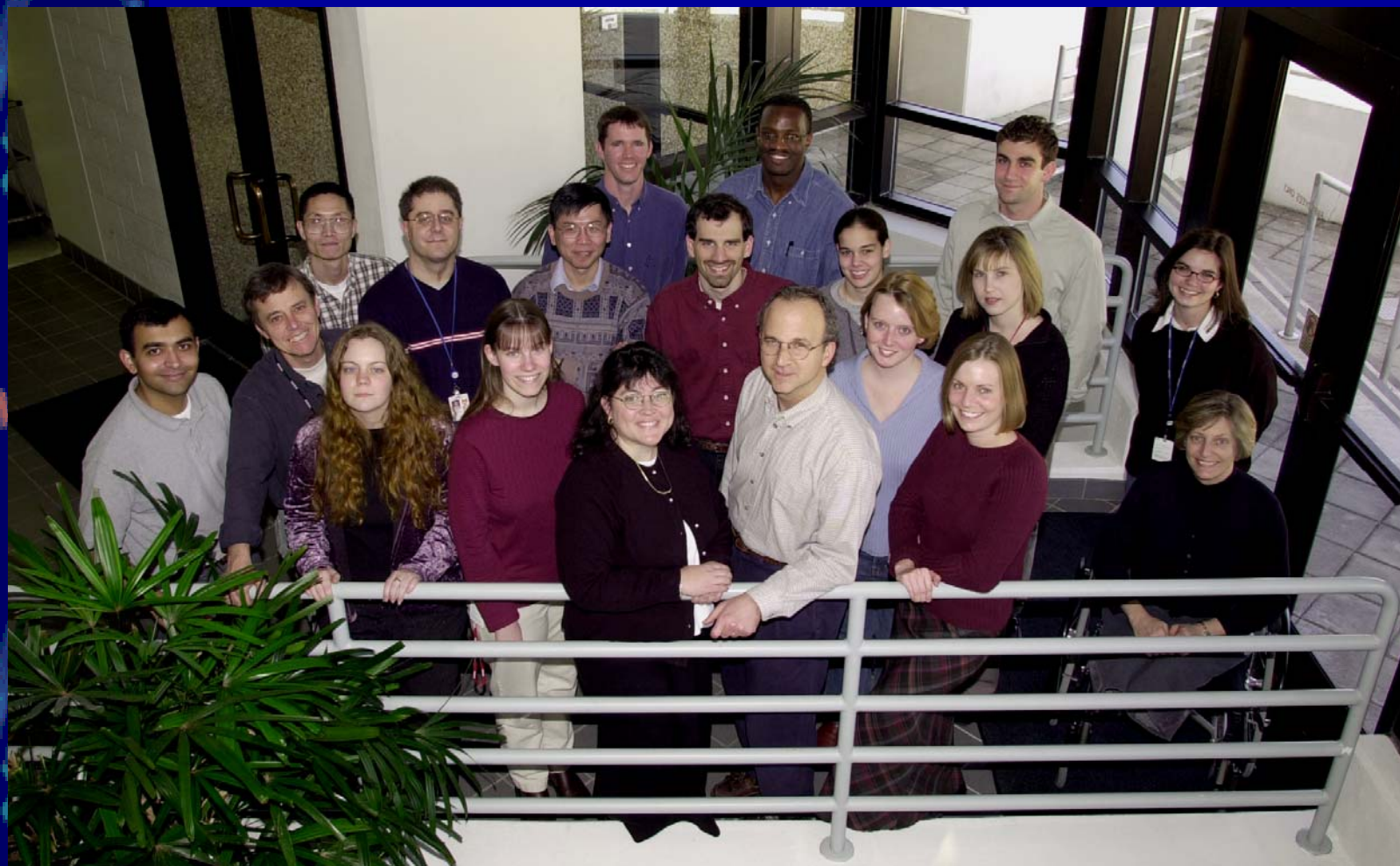


Summary

- Reducing RNA amounts
- Constantly Testing New Protocols
- Adding New Technologies such as 70mer Oligos



Acknowledgements





Acknowledgements

Pete Cozart

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